Leaf litter diversity and structure of microbial decomposer communities modulate litter decomposition in aquatic systems

Santschi, Fabienne ; Gounand, Isabelle ; Harvey, Eric ; Altermatt, Florian

Abstract: 1. Leaf litter decomposition is a major ecosystem process that can link aquatic to terrestrial ecosystems by flows of nutrients. Biodiversity and ecosystem functioning research hypothesizes that the global loss of species leads to impaired decomposition rates and thus to slower recycling of nutrients. Especially in aquatic systems, an understanding of diversity effects on litter decomposition is still incomplete. 2. Here we conducted an experiment to test two main factors associated with global species loss that might influence leaf litter decomposition. First, we tested whether mixing different leaf species alters litter decomposition rates compared to decomposition of these species in monoculture. Second, we tested the effect of the size structure of a lotic decomposer community on decomposition rates. 3. Overall, leaf litter identity strongly affected decomposition rates, and the observed decomposition rates matched measures of metabolic activity and microbial abundances. While we found some evidence of a positive leaf litter diversity effect on decomposition, this effect was not coherent across all litter combinations and the effect was generally additive and not synergistic. 4. Microbial communities, with a reduced functional and trophic complexity, showed a small but significant overall reduction in decomposition rates compared to communities with the naturally complete functional and trophic complexity, highlighting the importance of a complete microbial community on ecosystem functioning. 5. Our results suggest that top-down diversity effects of the decomposer community on litter decomposition in aquatic systems are of comparable importance as bottom-up diversity effects of primary producers.

DOI: https://doi.org/10.1111/1365-2435.12980

Originally published at:
Santschi, Fabienne; Gounand, Isabelle; Harvey, Eric; Altermatt, Florian (2018). Leaf litter diversity and structure of microbial decomposer communities modulate litter decomposition in aquatic systems. Functional Ecology, 32(2):522-532.
DOI: https://doi.org/10.1111/1365-2435.12980

Leaf litter diversity and structure of microbial decomposer communities modulate litter decomposition in aquatic systems

Fabienne Santschi[1,2], Isabelle Gounand[1,2], Eric Harvey[1,2] & Florian Altermatt[1,2,*]

[1] Eawag, Swiss Federal Institute of Aquatic Science and Technology, Department of Aquatic Ecology, Überlandstrasse 133, CH-8600 Dübendorf, Switzerland.
[2] Department of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland.

*corresponding author: Florian.Altermatt@eawag.ch

Number of words: 7300 (all, incl. References and legends)
Number of pages: 35
Number of figures: 5
Number of tables: 2

Abstract

1. Leaf litter decomposition is a major ecosystem process that can link aquatic to terrestrial ecosystems by flows of nutrients. Biodiversity and ecosystem functioning research hypothesizes that the global loss of species leads to impaired decomposition rates and thus to slower recycling of nutrients. Especially in aquatic systems an understanding of diversity effects on litter decomposition is still incomplete.
2. Here we conducted an experiment to test two main factors associated with global species loss that might influence leaf litter decomposition. Firstly, we tested whether mixing different leaf species alters litter decomposition rates compared to decomposition of these species in monoculture. Secondly, we tested the effect of the size structure of a lotic decomposer community on decomposition rates.
3. Overall, leaf litter identity strongly affected decomposition rates, and the observed decomposition rates matched measures of metabolic activity and microbial abundances. While we found some evidence of a positive leaf litter diversity effect on decomposition, this effect was not coherent across all litter combinations and the effect was generally additive and not synergistic.
4. Microbial communities, with a reduced functional and trophic complexity, showed a small but significant overall reduction in decomposition rates compared to communities with the naturally complete functional and trophic complexity, highlighting the importance of a complete microbial community on ecosystem functioning.

5. Our results suggest that top-down diversity effects of the decomposer community on litter decomposition in aquatic systems are of comparable importance as bottom-up diversity effects of primary producers.

Key words: *Alnus glutinosa,* Biodiversity ecosystem functioning, *Fagus sylvatica,* microcosm experiment, *Populus nigra*, protists, *Quercus robur*.

Introduction

Litter decomposition is a major process in nutrient recycling and plays an important role in the functioning of ecosystems (Hättenschwiler, Tiunov & Scheu 2005; Findlay 2012; Handa *et al.* 2014; García-Palacios *et al.* 2016; Bista *et al.* 2017). Plant detritus not only forms the vast majority of the dead organic matter pool in terrestrial systems, but is also an important source of energy in aquatic systems (Anderson & Sedell 1979). In aquatic systems, dead organic matter from plants can be generated *in situ* by aquatic vascular plants (i.e., autochthonous litter). However, *ex situ* (allochthonous) litter from tree leaves is often the more important source of organic matter (Fisher & Likens 1973; Gessner, Chauvet & Dobson 1999). Thereby, the surrounding terrestrial vegetation strongly affects both the composition and quantity of leaf litter input into aquatic systems (e.g., Hladyz *et al.* 2010; Hladyz *et al.* 2011), and such flows can even generate non-trivial linkages between ecosystems (Loreau, Mouquet & Holt 2003; Gravel *et al.* 2010; Harvey *et al.* 2016, 2017, Gounand *et al.*, 2017).

Recent work demonstrated that the decomposition of litter in lotic aquatic systems can be modulated by various factors related to litter type, decomposer and detritivore community type and general abiotic conditions (e.g., Lecerf *et al.* 2007; Woodward *et al.* 2012; Bruder *et al.* 2014; Frainer *et al.* 2015; Collins *et al.* 2016;

Stocker *et al*. 2017). As all of these main drivers of litter decomposition are affected by various environmental changes (e.g., Boyero *et al.* 2011; Frossard *et al.* 2013; Hines *et al.* 2014), understanding their independent and interactive effects on leaf litter decomposition and nutrient turnover is of high interest in order to predict the consequences of changes on ecosystem functioning (Handa *et al.* 2014).

The study of how litter diversity affects decomposition has especially attracted interest in terrestrial systems, with some studies showing an accelerated decomposition rate when increasing litter diversity (Wardle, Bonner & Nicholson 1997; Cardinale *et al.* 2011), while others finding no or even a negative relationships (for meta-analyses, see Gartner & Cardon 2004; Srivastava *et al.* 2009). As mentioned, however, a significant portion of terrestrial litter decomposition is occurring in aquatic systems (Ball *et al.* 2010). Surprisingly, in aquatic ecosystems the focus has often been on effects of leaf litter quality, climate or the structure of the decomposer community (e.g., Frossard *et al.* 2013; Frainer *et al.* 2015; García-Palacios *et al.* 2016; Hines, Reyes & Gessner 2016) on decomposition rates, rather than on effects of litter diversity *per se* (but see e.g., Gessner *et al.* 2004; Giller *et al.* 2004; Handa *et al.* 2014). Consequently, the specific effects of leaf litter diversity and identity and the decomposer community in aquatic systems are still not completely resolved and have been proposed to be to some degree system dependent (Hättenschwiler, Tiunov & Scheu 2005; Cardinale *et al.* 2011; Lecerf *et al.* 2011). Furthermore, in aquatic ecosystems, leaf litter decomposition can be controlled both by bottom-up (litter diversity, see Gessner *et al.* 2004; Giller *et al.* 2004; Handa *et al.* 2014; García-Palacios *et al.* 2016) and top-down (Srivastava & Bell 2009; Srivastava *et al.* 2009) processes, and a synthesis of their relative role has not yet emerged (Giller *et al.* 2004).

Here, we studied how the diversity and identity of allochthonous leaf litter from common tree species and the size structure of a natural aquatic microbial decomposer community extracted from a lotic system (small, dammed forest stream; see Fig. S1 in Supporting information) affect litter decomposition in aquatic ecosystems. To achieve this goal, we used four leaf litter species (alder, beech, poplar and oak; Fig. 1) in experimental mono-, bi- and poly-cultures, and exposed them to decomposition by a natural aquatic microbial community and a microbial community of which we manipulated the size structure by excluding larger, potentially predatory, eukaryotic microbial organisms. We followed decomposition of leaves and tracked microbial activity (oxygen concentration) and community dynamics of free-living microbes (density and size structure of bacteria and protists) to functionally link the structure of the microbial decomposer community and leaf litter diversity to the process of litter decomposition. Our approach explicitly allowed us to address both bottom-up diversity effects of leaf litter as well as top-down diversity effects of decomposer organisms on decomposition.

Methods

General experimental set-up

We tested the effects of leaf litter quality and diversity and the structural complexity of the decomposer community on litter decomposition in a microcosm laboratory experiment. We used leaf litter from four tree species common and native to Central Europe that display a range of litter quality: black alder (*Alnus glutinosa)*, European beech (*Fagus sylvatica)*, black poplar (*Populus nigra)* and pedunculate oak (*Quercus robur)*; in the following we refer to these four species using their genus name. We selected these species as *Alnus* and *Populus* are considered to be good quality resources, while *Quercus* and *Fagus* are known to be generally of lower quality (see for example Hladyz *et al.* 2009; Frainer *et al.* 2015). We used naturally senesced, air-dried leaves. Previous to the experiment, the leaves from all four species were mixed together and leached in river water for 24 hours so that water-soluble and possibly inhibitory compounds in the leaves (e.g., tannins) could leach out. We then cut leaf discs (ø = 2.5 cm) from all leaf species and dried them for 60 hours in a drying oven. The leaf discs were then individually weighed. We used a subset of leaves from the same batch as used in the experiment and analysed them for carbon, nitrogen, and phosphorus content using established protocols (phosphorus: San++ automated wet chemistry analyzer, Skalar Analytical B.V., Breda, Netherlands; nitrogen and carbon: Flash 2000 Elemental Analyzer coupled with Delta V Advantage IRMS, both manufactured by ThermoFisher Scientific, Bremen, Germany). The values reported from these measurements in table 1 are the same as also reported in Little & Altermatt (in review).

In each microcosm we placed a total of four leaf discs of different species combinations: microcosms contained either a single leaf litter species (i.e., four leaf discs of either *Alnus*, *Fagus*, *Populus* or *Quercus* respectively), mixtures of two leaf litter species (i.e., two leaf discs of two leaf species, in all possible pairwise combinations) or leaf discs of all four species (i.e., one leaf disc from each species), resulting in 11 different leaf litter treatments (Fig. 1).

We used natural aquatic microbial decomposer communities of two different structural complexities to test for possible interactive effects of the decomposer community trophic structure with litter diversity. Natural microbial communities originated from a small, dammed stream surrounded by deciduous forest near Pfäffikon ZH, Switzerland (location: 47° 22’ 27.1” North, 8° 48’ 08.3” East) (see also Mächler & Altermatt 2012). We sampled the water including the microbial communities near the inflow (Fig. S1 in Supporting Information), such that our study looks at water and microbial decomposers that are characteristic of a lotic system. Twenty liters of water was sampled in October 2015 and filtered on site to remove large aquatic organisms such as macroinvertebrates or vertebrate larvae (mesh size 250 µm). The filtered water contained the natural microbial decomposer community consisting of bacteria, fungi and protists, and henceforth is referred to as the "complete decomposer community" ("CDC"). To obtain a size-fractionated community ("SFC") with a reduced functional and trophic complexity (i.e., exclusion of large organisms such as predatory rotifers or ciliates), we filtered half of the water through a much finer filter (mesh size 11 µm). Many of these microbial organisms are rather flexible in their body structure (e.g., amoeba which can change their shape very plastically and have substantial intraspecific variability in size, see Giometto *et al.*, 2013), and thus the 11 µm filter is not a clear-cut threshold: some organisms may pass when small, but grow bigger thereafter, or some organisms are much longer than 11 µm, but very slender, and can thus still pass. Overall, however, the filtering significantly reduced the abundance and occurrence of organisms larger than 10 µm (linear mixed effect model, $p < 0.001$), thus proving the effectiveness of the filtering.

While focusing here on bacteria and protists, we recognize the important role of fungi for decomposition processes in lotic systems (e.g., Gessner & Chauvet 1994; Hieber & Gessner 2002; Dang, Chauvet & Gessner 2005; Gessner *et al.* 2007). To ensure that microbial (i.e. also fungal) colonization of leaves could occur, all leaves were conditioned in one vessel filled with stream water for 24 h. Furthermore, microbial communities, including fungal spores, came in through the water sampled from the dammed forest stream and used for the experiment. We could, however, not measure fungal components in the leaf biomass for logistic and technical reasons.

Importantly, however, our goal was to study the effect of leaf litter identity and decomposer community size structure, but not community identity of the latter.

All microcosms were filled with 100 mL of the corresponding decomposer community (CDC versus SFC), with five replicates per treatment combination, resulting in a total of 110 microcosms (Fig. 1). Microcosms were filled with the different resource types (leaves) and the corresponding decomposer community on 27th October 2015 and leaf litter was subsequently incubated in these aquatic microcosms for a decomposition period of 72 days. The experiment took place in a climate room with a constant temperature of 18±1 °C and a day/night-cycle of 12 h light and 12 h darkness. All handling and work was conducted using standard microbiology procedures, including sterile handling procedures and autoclaving all material (such as pipettes, glassware etc.) previous to its use. Cultures were regularly screened visually with a stereomicroscope (Leica M205 C, Leica Microsystems, Heerbrugg, Switzerland) at a 10 to 160-fold magnification, using dark-field illumination. Further general handling and laboratory procedures for such aquatic microcosms are described in detail in Altermatt *et al*. (2015).

Response variables

Our primary response variable was leaf biomass loss (as a proxy for decomposition rates). Oxygen concentration and the composition and structure of bacteria and protist communities were used as complementary response variables underlying drivers of decomposition/decomposer activity.

To measure leaf biomass loss, we removed the leaf discs after 72 days of incubation and carefully cleaned them from the biofilm under running tap water. We then dried the leaf discs at 60 °C for 60 hours and measured the final dry mass of all individual leaf discs.

We measured dissolved oxygen concentrations in the microcosms every two days during the first four weeks of the experiment and thereafter for organizational reasons twice a week for the remaining six weeks with an optical oxygen meter (PreSens Fibox 4 Optical Oxygen Meter). Oxygen concentration is often negatively correlated with microbial activity, and can in parts be used as a proxy of it (Briand *et al.* 2004). Importantly however, in our case there were also likely photosynthetic organisms present, such that microbial activity could to some degree also increase O_2 levels. While we did not see a pronounced development of a photosynthetic biofilm, the longer term dynamics in O_2 concentrations likely included a combination and equilibrium between O_2 consumption during decomposition and O_2 production by phototrophic organisms. We thus see the O_2 measurements reflecting microbial activities in a broader sense.

We measured density and cell size distributions of free-living protists and other microorganisms (e.g., rotifers) with a diameter >5 µm in the decomposer communities with a Cell Counter and Analyzer System (CASY) model TTC (Roche Diagnostics GmbH) at weekly intervals during the experiment (Mächler & Altermatt 2012; Altermatt *et al.* 2015). We took 0.5 mL samples and diluted them 1:20 with the isotonic buffer solution CASYTon®. Cell counts were performed with a 150 µm capillary, and individual cell counts and cell size measurements were used to estimate the total biomass of decomposers in the microcosms (Giometto *et al.* 2013; Altermatt *et al.* 2015).

Finally, we measured abundance of bacteria with a BD Accuri™ C6 flow cytometer (Becton-Dickinson) during the experiment at roughly one-week intervals.

Samples were diluted with filtered Evian® according to expected densities within the microcosms, stained with 20 µl of the fluorescent dye SYBR® Green and incubated for 13 minutes at 37 °C. The measurements were made from 50 µL samples and a threshold value of 800 on FL1-H (green fluorescence level). We used well-established gating settings to distinguish between background noise and bacterial counts (Altermatt *et al.* 2015).

Data Analysis

We used the *R* software version 3.3.2 (R Development Core Team 2016) for all statistical analyses. We calculated the proportion of the final leaf litter dry weight compared to the initial leaf litter dry weight as the decomposition rate (odds ratio). We used generalized linear models (GLMs) with quasi-binomial link functions to examine the influence of our predictor variables, resource type and decomposer community type, on leaf mass loss. To disentangle the effects of the different resource types we conducted *post-hoc* multiple linear pairwise Tukey-test comparisons using the R-package 'multcomp' (Hothorn *et al.* 2016).

For the proximate response variables, we used linear mixed effect models in the R-package 'lmerTest' (Kuznetsova, Brockhoff & Christencesn 2015) to test the effects of leaf litter diversity and consumer community on oxygen concentrations, total cell counts, living biomass, median organism size and bacterial densities in the community. The resource type and the decomposer community were used as fixed effects whereas time was used as a random effect.

Results

Leaf litter decomposition differed significantly between litter types and combinations thereof, and between the two decomposer community types (Fig. 2 and table 2). There was no interaction between leaf litter treatment and decomposer community structure. In all treatments, *Populus* and *Alnus* leaves were more strongly decomposed than *Fagus* and *Quercus* leaves, and most of these differences were significant or marginally significant (decomposition *Populus* > *Fagus*, $p < 0.001$; decomposition *Populus* > *Quercus*, $P < 0.001$; decomposition *Populus* > *Alnus*, $p = 0.03$; decomposition *Alnus* > *Fagus*, $p= 0.08$; decomposition *Alnus* > *Quercus*, $p = 0.07$; decomposition *Fagus* ~ *Quercus*, $p = 0.97$; Figs. 2 & 3, complete statistical details are given in table S1 in Supporting Information). Size-fractionated communities showed a small but significant reduction in decomposition rates compared to complete communities, which included higher trophic levels and larger organisms (Fig. 2, table 2). Overall, the most common effect of mixing different leaf types on decomposition rates was additive, but we also found some synergistic effects (the expected value is the mean of the two species' values in monoculture and denoted by the red line in Fig. 2; the observed value, indicated by the bar, is in some cases higher than the expected value; see tables A2 & A4 for full overview of statistical results). When looking at decomposition rates of each leaf litter species individually, we found no differences in decomposition for leaves of *Fagus*, *Populus* or *Quercus* when decomposed alone compared to in mixture with other species (all $p > 0.05$; Fig. 3b–d & 3f–h; tables S2, S3 & S4 in Supporting Information). In stark contrast, *Alnus* leaves decomposed at significantly higher rates when mixed with other leaf species ($p < 0.0002$; Fig. 3a & 3e, table S5 in Supporting Information).

Oxygen concentrations showed pronounced temporal dynamics with a drastic decrease in the first five days, and a subsequent increase to a stable value after about 30 days. We found highly significant effects of leaf litter type on O_2 concentration and significantly lower O_2 concentrations in the complete *vs.* size-fractionated communities (Fig. 4 and table 3). The mixing of leaf litter generally resulted in intermediate O_2 concentrations compared to single leaf litter treatments (i.e., additive effects on O_2 concentration, Fig. S3 to S8 in Supporting Information).

Leaf litter type also significantly influenced microbial cell counts (eukaryotic and prokaryotic) and total microbial biomass (Fig. 5 and table 3). As expected, filtering communities initially with a 11 µm filter removed and significantly reduced organisms >10 µm in SFC compared to CDC ($p < 0.01$). The removal of the larger organisms resulted in a marginally significantly lower median organism size in the size-fractionated community compared to the whole microbial community (table 3). Median size increased in all treatments consistently over time. Surprisingly, decreasing structural (i.e., size) complexity of the communities did not significantly affect proximate microbial community structures over time (Fig. 5), even though the ultimate effects on decomposition were detectable and significant (see above). Initially, microbial abundance increased in microcosms containing leaves of *Populus* or *Alnus* (in both microbial community types) and of *Quercus* (only in the SFC; Fig. 5a/b). After this initial peak, abundances decreased and stabilized to a constant value after 30 days. The abundance of microbes in microcosms containing *Fagus* was low during the whole decomposition process. Mixing leaf litter mostly resulted in intermediate values of cell counts (additive effects of leaf mixture, data not shown). Biomass of the microbial community at the end of the experiment was highest in microcosms containing *Quercus*, followed by *Alnus*, *Populus* and *Fagus*. Similarly, the median of organisms' cell size distribution steadily and significantly increased over time in the decomposer communities (Fig. 5e/f), although without a significant difference between the leaf litter treatments (table 3).

In contrast to these overall microbial community shifts, bacterial densities significantly declined over time in all treatment combinations (Fig. S2 Supporting Information), with significant differences between leaf litter treatments but no significant effect of initial community structure (table 3). There was no consistent influence of mixing leaf litter on bacterial abundances, but often they were intermediate compared to the single leaf-litter treatments (additive effects of leaf mixture, data not shown).

Discussion

We found that leaf litter identity strongly influenced litter decomposition rates, but that rates were also modulated by the structural composition of the free-living decomposer community. Consistent with previous work in stream systems, mixing leaf litter generally exhibited an additive rather than a synergistic effect on decomposition (e.g., Kominoski *et al.* 2007). Additionally, we found that manipulating the size structure of the decomposer community has a direct influence on decomposition rates and on biological processes (microbial activity as measured by O_2 concentration), while some of the proximate measures of community structure were not significantly affected. Specifically, a complete decomposer community showed faster decomposition compared to the sized-fractionated decomposer community. The size-fractionated communities were not only lacking larger organisms due to the filtering (size threshold of the filtration was about 10–15 μm), but the whole community overall consisted of marginally significantly smaller organisms. The removal of larger organisms likely resulted also in a removal of trophically higher microbes, such as predatory rotifers or ciliates, or other specific functional types of organisms. The predominant absence of synergistic litter diversity effect on free-living aquatic decomposition rates may render interpretations and extrapolations of decomposition rates more predictable, as the majority of effects was additive.

Leaf Litter Decomposition

Leaf litter identity and associated traits are a crucial factor affecting rates of litter decomposition in aquatic systems (Webster & Benfield 1986; Lecerf *et al.* 2007; Gessner *et al.* 2010; Bruder *et al.* 2014). Thereby, both the content and ratio of C, N and P as well as lignin are important determinants of leaf litter decomposition. Generally, the higher the N-content (or the N content relative to the C content), the better leaves can be decomposed. Our observed decomposition rates are in good accordance to the measured C:N ratios (table 1), and the P- and N-content of the leaves: C:N ratio was *Quercus* ~ *Fagus* > *Populus* > *Alnus*, which matched (expect for *Populus* and *Alnus* reversed in most cases) the decomposition rates. In analogy, the more lignin a leaf contains, the slower its decomposition (Hladyz *et al.* 2009; Schindler & Gessner 2009; Frainer *et al.* 2015). Our findings of decomposition rates are consistent when comparing them to lignin contents of our leaf species derived from literature data: *Fagus* and *Quercus*, which are generally having highest lignin contents (e.g., Hladyz *et al.* 2009; Frainer *et al.* 2015), were decomposed the slowest. In contrast, *Populus* with a generally low lignin content (e.g., Frainer *et al.* 2015) was decomposed the fastest. *Alnus* has intermediate, but rather variable lignin contents (e.g., Hladyz *et al.* 2009; Frainer *et al.* 2015) and—depending on the decomposer community structure—were decomposed either as well as *Populus* or as slowly as *Fagus* and *Quercus*.

So far, various effects of leaf litter diversity on decomposition rates were found, including additive (Srivastava *et al.* 2009; Frainer *et al.* 2015) and synergistic effects (Lecerf *et al.* 2011; Handa *et al.* 2014). Importantly, these studies cover different ecosystems, from lentic to lotic ecosystems, and also different leaf types/leaf species and conditioning. Overall, recent studies in lotic systems, where decomposition by fungi is found important (e.g., Gessner & Chauvet 1994; Hieber & Gessner 2002; Dang, Chauvet & Gessner 2005; Gessner *et al.* 2007), fairly consistently report a lack of a synergism (Ferreira, Encalada & Graça 2012; Bruder *et al.* 2014), suggesting that leaf identity might be a more important factor than litter diversity in determining decomposition rates. While we could not measure fungi themselves, but focused on the free-living decomposer community present in the supernatant, our results are in high concordance with these findings, and the observed additive effects of mixing leaf litter could arise from two different mechanisms. Either the component species get degraded at the same rate in mixtures as in monocultures, or mixing leaf litter affected the decomposition of the two component leaf litter species in opposing directions, with the sum of overall decomposition resulting in an overall additive effect. While *Alnus* leaves decomposed differently depending on the co-occurring leaves (Fig. 3a,e), we found that leaves of *Fagus*, *Populus* and *Quercus* did not decompose differently when mixed with other species (Figs. 3b–d & 4f–h; tables A2 & S4 Supporting Information). Thus, we found differences in decomposition of leaves in some combinations, while not in other combinations. Constant decomposition rates of a focal species when mixed with other species had also been previously observed (Ferreira, Encalada & Graça 2012; Bruder *et al.* 2014). This would provide some support for the first mechanism, that leaf litter gets degraded with a constant rate regardless of the presence of other species. Importantly, however, these past studies focused on the effect of fungi on decomposing leaves, while we could not measure fungi themselves. Thus, our results need to be interpreted with some care when being compared to these other studies.

As mentioned above, we also found strong exceptions to this overall additive effect of mixing leaf litter species (Fig. 4). When mixing *Fagus* or *Quercus* with *Alnus* leaves, we observed higher overall decomposition than the expected average of the two component species (Fig. 2, AF AQ and AFPQ treatments; tables A1 & A3 Supporting Information). In our experiment we observed these non-additive effects only when mixing a low quality leaf litter (i.e., *Fagus* and *Quercus* with a low nitrogen content; table 1) with a high quality leaf litter (especially *Alnus* with a high nitrogen content; table 1) (see also Vos *et al.* 2013). In addition, *Fagus* also had the lowest phosphorus content (table 1) and is generally reported to have a high lignin content (Frainer *et al.* 2015), making it the most dissimilar leaf quality type relative to *Alnus*. As a possible consequence, the diversity effect was most pronounced when mixing *Alnus* with *Fagus*, indicating that dissimilarities in leaf litter qualities are clearly a prerequisite for accelerated decomposition rates. While not explicitly studied (and not addressable with our study design), this could indicate some support of a functional diversity effect.

Proximate effects on microbial and bacterial communities

Leaf litter identity strongly influenced O_2 concentrations in the microcosms (Fig. 5) and the observed O_2 concentrations during the early phase of the experiment closely matched the inverse of overall decomposition rates. The strong temporal fluctuations with an initial decrease in O_2 concentrations, and a subsequent increase and then steady state could be explained by a combination of depletion of nutrients (Dilly & Munch 1996) resulting in lower decomposer activities during the latter half of the experiment (and O_2 diffusing into the medium), the potential formation of a photosynthetically active biofilm, in which microbial activity was not only consuming but also producing O_2, or the presence of leachates and inhibitory compounds during the initial phase and an associated community turn-over during the experiment from fungi to bacteria dominance. Initial colonization and decomposition of the leaves results in a rapid decomposition of the more labile compounds, while more recalcitrant compounds can only be accessed later on.

Microbial cell counts, representing the number of free-living eukaryotic organisms such as protists, showed as expected the inverse pattern to oxygen concentrations (Fig. 5): an initial increase of organisms could be detected, but then the number of organisms decreased. Bacterial densities also declined over time (Fig. S2 Supporting Information). This is consistent with an initial high availability of nutrients but subsequent depletion. Surprisingly, however, the total biomass increased steadily over time (Fig. 5), paralleled by an increase in the median cell size of the community over time (Fig. 5). This suggests a shift in the community structure towards fewer larger organisms.

In the complete decomposer community, larger, possibly bacterivorous, protists were likely present, which are expected to substantially reduce bacteria abundances. As a consequence, we expected lower decomposition rates. However, we found the opposite result. This counterintuitive increase in decomposition rates in the presence of larger bacterivorous/predatory protists has also been seen in other studies (Barsdate & Prenski 1974, Ribblett *et al.* 2005), and has been explained by a high turnover of bacteria leading to a better physical state of the bacterial community consequently enhancing decomposition. We see three mutually non-exclusive explanations. First, it could be a top-down effect of the larger microorganisms (“meiofauna”) on the smaller decomposers. However, in our case bacterial densities did not vary with the structure of the decomposer community (CDC *vs.* SFC), arguing against this positive effect of grazing. Second, the meiofauna itself may not only consist of predators, but also include some decomposers. Thus, the meiofauna would to some level increase predation but also increase decomposition. In that case, the complete decomposer community would actually also include a potentially higher diversity of leaf consumers. Finally, it could also indicate a distinct enzymatic capacity towards more recalcitrant compounds. A meta-analysis indeed provided evidence for a *per se* positive relationship between consumer diversity (decomposer community) and decomposition rates (Srivastava *et al.* 2009). Such a diversity effect at the decomposer level can result from several mechanisms. First, facilitation among microorganisms can occur during the process of litter decomposition (De Boer *et al.* 2005). Additionally, complementary resource use can ensue (Gessner *et al.* 2010), resulting in the break-down of a wider range of leaf litter components. The latter mechanism though can only occur if species are functionally diverse. Our experiment showed a pronounced positive effect of trophic complexity in microbial communities on leaf litter decomposition rates (see also Handa *et al.* 2014). Whether this is a consequence of species richness or functional diversity is challenging to unravel, because by reducing the functional diversity via size-fractioning the community, we simultaneously reduced species richness. Overall, our results underpin that the trophic complexity of a decomposer community (e.g., see also Stocker *et al.* 2017), also at the microbial level, is crucial for the functioning of the litter decomposition process.

Conclusion

We found that leaf litter identity and quality significantly and strongly influence decomposition rates. Only in the case of *Alnus* and *Fagus*, mixing leaf litter species resulted in synergistic effects in decomposition rates. For the other species combinations, the effects were additive. This suggests that the diversity of primary producers is not as important in the process of litter decomposition as in other ecosystem functions, such as primary production. Importantly, decomposition rates were higher in microbial decomposer communities that were not size-fractionated compared to microbial decomposer communities in which medium to large-sized microbes were initially removed, even though many of our metrics characterizing these communities (e.g., size structure, abundance etc.) were surprisingly similar throughout the experiment. This finding implies that trophic diversity and functional traits of the decomposer community are important for litter decomposition and subsequent nutrient cycling. Overall, top-down effects due to loss of species or functional groups in the decomposer community may be as important as bottom-up effects via leaf litter (i.e., resource) diversity highlighting the sensitivity of decomposition processes to future environmental changes.

Author Contributions

All authors planned and designed the study; FS conducted the experiment and collected the data; FS and FA analyzed the data. FS and FA led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Acknowledgements

We thank Sereina Gut for help during the field and laboratory work and Chelsea J. Little for providing the leaf litter used in the experiment and the calculation of the leaf composition values, for discussions and for giving comments on the manuscript. We thank Daniel Steiner for the chemical analysis of the leaf litter. We thank two anonymous reviewers for constructive comments on the manuscript. Funding is from the Swiss National Science Foundation Grant No PP00P3_150698 (to F.A.)

Data accessibility

All data from the study will be archived on Dryad upon acceptance of the manuscript.

References

Altermatt, F., Fronhofer, E.A., Garnier, A., Giometto, A., Hammes, F., Klecka, J., Legrand, D., Mächler, E., Massie, T.M., Pennekamp, F., Plebani, M., Pontarp, M., Schtickzelle, N., Thuillier, V. & Petchey, O.L. (2015) Big answers from small worlds: a user's guide for protist microcosms as a model system in ecology and evolution. *Methods in Ecology and Evolution,* **6,** 218-231.

Anderson, N.H. & Sedell, J.R. (1979) Detritus processing by macroinvertebrates in stream ecosystems. *Annual Review of Entomology,* **24,** 351-377.

Baldy, V., Gessner, M.O. & Chauvet, E. (1995) Bacteria, Fungi and the Breakdown of Leaf Litter in a Large River. *Oikos,* **74,** 93-102.

Ball, B.A., Kominoski, J.S., Adams, H.E., Jones, S.E., Kane, E.S., Loecke, T.D., Mahaney, W.M., Martina, J.P., Prather, C.M., Robinson, T.M.P. & Solomon, C.T. (2010) Direct and Terrestrial Vegetation-Mediated Effects of Environmental Change on Aquatic Ecosystem Processes. *BioScience,* **60,** 590-601.

Bista, I., Carvalho, G.R., Walsh, K., Seymour, M., Hajibabaei, M., Lallias, D., Christmas, M. & Creer, S. (2017) Annual time-series analysis of aqueous eDNA reveals ecologically relevant dynamics of lake ecosystem biodiversity. *Nature Communications,* **8,** 14087.

Boyero, L., Pearson, R.G., Gessner, M.O., Barmuta, L.A., Ferreira, V., Graça, M.A.S., Dudgeon, D., Boulton, A.J., Callisto, M., Chauvet, E., Helson, J.E., Bruder, A., Albariño, R.J., Yule, C.M., Arunachalam, M., Davies, J.N., Figueroa, R., Flecker, A.S., Ramírez, A., Death, R.G., Iwata, T., Mathooko, J.M., Mathuriau, C., Gonçalves, J.F., Moretti, M.S., Jinggut, T., Lamothe, S., M'Erimba, C., Ratnarajah, L., Schindler, M.H., Castela, J., Buria, L.M., Cornejo, A., Villanueva, V.D. & West, D.C. (2011) A global experiment suggests climate warming will not accelerate litter decomposition in streams but might reduce carbon sequestration. *Ecology Letters,* **14,** 289-294.

Briand, E., Pringault, O., Jacquet, S. & Torréton, J.P. (2004) The use of oxygen microprobes to measure bacterial respiration for determining bacterioplankton growth efficiency. *Limnology and Oceanography: methods,* **2,** 406-416.

Bruder, A., Schindler, M.H., Moretti, M.S. & Gessner, M.O. (2014) Litter decomposition in a temperate and a tropical stream: the effects of species mixing, litter quality and shredders. *Freshwater Biology,* **59,** 438-449.

Cardinale, B.J., Matulich, K.L., Hooper, D.U., Byrnes, J.E., Duffy, E., Gamfeldt, L., Balvanera, P., O'Connor, M.I. & Gonzalez, A. (2011) The functional role of producer diversity in ecosystems. *American Journal of Botany,* **98,** 572-592.

Collins, S.M., Kohler, T.J., Thomas, S.A., Fetzer, W.W. & Flecker, A.S. (2016) The importance of terrestrial subsidies in stream food webs varies along a stream size gradient. *Oikos,* **125,** 674-685.

Dang, C.K., Chauvet, E. & Gessner, M.O. (2005) Magnitude and variability of process rates in fungal diversity-litter decomposition relationships. *Ecology Letters,* **8,** 1129–1137.

Ferreira, V., Encalada, A.C. & Graça, M.A.S. (2012) Effects of litter diversity on decomposition and biological colonization of submerged litter in temperate and tropical streams. *Freshwater Science,* **31,** 945-962.

Findlay, S.E.G. (2012) Organic matter decomposition. *Fundamentals of Ecosystem Science,* (eds K.C. Weathers, D.L. Strayer & G.E. Likens). Elsevier, London.

Fisher, S.G. & Likens, G.E. (1973) Energy Flow in Bear Brook, New Hampshire: An Integrative Approach to Stream Ecosystem Metabolism. *Ecological Monographs,* **43,** 421-439.

Frainer, A., Moretti, M.S., Xu, W. & Gessner, M.O. (2015) No evidence for leaf-trait dissimilarity effects on litter decomposition, fungal decomposers, and nutrient dynamics. *Ecology,* **96,** 550-561.

Frossard, A., Gerull, L., Mutz, M. & Gessner, M.O. (2013) Litter Supply as a Driver of Microbial Activity and Community Structure on Decomposing Leaves: a Test in Experimental Streams. *Applied and Environmental Microbiology,* **79,** 4965-4973.

García-Palacios, P., McKie, B.G., Handa, I.T., Frainer, A. & Hättenschwiler, S. (2016) The importance of litter traits and decomposers for litter decomposition: a comparison of aquatic and terrestrial ecosystems within and across biomes. *Functional Ecology,* **30,** 819-829.

Gartner, T.B. & Cardon, Z.G. (2004) Decomposition dynamics in mixed-species leaf litter. *Oikos,* **104,** 230-246.

Gessner, M.O. & Chauvet, E. (1994) Importance of Stream Microfungi in Controlling Breakdown Rates of Leaf Litter. *Ecology,* **75,** 1807-1817.

Gessner, M.O., Chauvet, E. & Dobson, M. (1999) A perspective on leaf litter breakdown in streams. *Oikos,* **85,** 377-384.

Gessner, M.O., Gulis, V., Kuehn, K.A., Chauvet, E. & Suberkropp, K. (2007) Fungal decoposers of plant litter in aquatic ecosystems. *Environmental and Microbial*

Relationships (eds C.P. Kubicek & I.S. Druzhiina), pp. 301–324. Springer, Berlin.

Gessner, M.O., Inchausti, P., Persson, L., Raffaelli, D.G. & Giller, P.S. (2004) Biodiversity effects on ecosystem functioning: insights from aquatic systems. *Oikos,* **104,** 419-422.

Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H. & Haettenschwiler, S. (2010) Diversity meets decomposition. *Trends in Ecology & Evolution,* **25,** 372-380.

Giller, P.S., Hillebrand, H., Berninger, U.G., Gessner, M.O., Hawkins, S., Inchausti, P., Inglis, C., Leslie, H., Malmqvist, B., Monaghan, M.T., Morin, P.J. & O'Mullan, G. (2004) Biodiversity effects on ecosystem functioning: Emerging issues and their experimental test in aquatic environments. *Oikos,* **104,** 423-436.

Giometto, A., Altermatt, F., Carrara, F., Maritan, A. & Rinaldo, A. (2013) Scaling body size fluctuations. *Proceedings of the National Academy of Sciences,* **110,** 4646-4650.

Gounand, I., Harvey, E., Ganesanandamoorthy, P. & Altermatt, F. (2017) Subsidies mediate interactions between communities across space. *Oikos*, **126**, 972-979.

Gravel, D., Guichard, F., Loreau, M. & Mouquet, N. (2010) Source and sink dynamics in meta-ecosystems. *Ecology,* **91,** 2172-2184.

Handa, I.T., Aerts, R., Berendse, F., Berg, M.P., Bruder, A., Butenschoen, O., Chauvet, E., Gessner, M.O., Jabiol, J., Makkonen, M., McKie, B.G., Malmqvist, B., Peeters, E.T.H.M., Scheu, S., Schmid, B., van Ruijven, J., Vos, V.C.A. & Hattenschwiler, S. (2014) Consequences of biodiversity loss for litter decomposition across biomes. *Nature,* **509,** 218-221.

Harvey, E., Gounand, I., Ganesanandamoorthy, P. & Altermatt, F. (2016) Spatially cascading effect of perturbations in experimental meta-ecosystems. *Proceeding of the Royal Society B-Biological Sciences,* **283,** 20161496.

Harvey, E., Gounand, I., Little, C.J., Fronhofer, E.A. & Altermatt, F. (2017) Upstream trophic structure modulates downstream community dynamics via resource subsidies. *Ecology and Evolution,* **7**, 5724–5731.

Hättenschwiler, S., Tiunov, A.V. & Scheu, S. (2005) Biodiversity and litter decomposition in terrestrial ecosystems. *Annual Review of Ecology, Evolution and Systematics,* **36,** 191-218.

Hieber, M. & Gessner, M.O. (2002) Contribution of stream detrivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology,* **83,** 1026-1038.

Hines, J., Reyes, M. & Gessner, M.O. (2016) Density constrains cascading consequences of warming and nitrogen from invertebrate growth to litter decomposition. *Ecology,* **97,** 1635-1642.

Hines, J., Reyes, M., Mozder, T.J. & Gessner, M.O. (2014) Genotypic trait variation modifies effects of climate warming and nitrogen deposition on litter mass loss and microbial respiration. *Global Change Biology,* **20,** 3780-3789.

Hladyz, S., Abjornsson, K., Chauvet, E., Dobson, M., Elosegi, A., Ferreira, V., Fleituch, T., Gessner, M.O., Giller, P.S., Gulis, V., Hutton, S.A., Lacoursiere, J.O., Lamothe, S., Lecerf, A., Malmqvist, B., McKie, B.G., Nistorescu, M., Preda, E., Riipinen, M.P., Risnoveanu, G., Schindler, M., Tiegs, S.D., Vought, L.B.M. & Woodward, G. (2011) Stream Ecosystem Functioning in an Agricultural Landscape: The Importance of Terrestrial-Aquatic Linkages. *Advances in Ecological Research, Vol 44* (ed. G. Woodward), pp. 211-276.

Hladyz, S., Gessner, M.O., Giller, P.S., Pozo, J. & Woodward, G.U.Y. (2009) Resource quality and stoichiometric constraints on stream ecosystem functioning. *Freshwater Biology,* **54,** 957-970.

Hladyz, S., Tiegs, S.D., Gessner, M.O., Giller, P.S., Risnoveanu, G., Preda, E., Nistorescu, M., Schindler, M. & Woodward, G. (2010) Leaf-litter breakdown in pasture and deciduous woodland streams: a comparison among three European regions. *Freshwater Biology,* **55,** 1916-1929.

Hothorn, T., Bretz, F., Wetsfall, P., Heiberger, R.M., Schuetzenmeister, A. & Scheibe, S. (2016) "multcomp": Simultaneous Inference in General Parametric Models.

Kominoski, J.S., Pringle, C.M., Ball, B.A., Bradford, M.A., Coleman, D.C., Hall, D.B. & Hunter, M.D. (2007) Nonadditive effects of leaf litter species diversity on breakdown dynamics in a detritus-based stream. *Ecology,* **88,** 1167-1176.

Kuznetsova, A., Brockhoff, P.B. & Christencesn, R.H.B. (2015) Package "lmerTest": Tests in Linear Mixed Effects Models.

Lecerf, A., Marie, G., Kominoski, J.S., LeRoy, C.J., Bernadet, C. & Swan, C.M. (2011) Incubation time, functional litter diversity, and habitat characteristics predict litter-mixing effects on decomposition. *Ecology,* **92,** 160-169.

Lecerf, A., Risnoveanu, G., Popescu, C., Gessner, M.O. & Chauvet, E. (2007) Decomposition of diverse litter mixtures in streams. *Ecology,* **88,** 219-227.

Loreau, M., Mouquet, N. & Holt, R.D. (2003) Meta-ecosystems: a theoretical framework for a spatial ecosystem ecology. *Ecology Letters,* **6,** 673-679.

Mächler, E. & Altermatt, F. (2012) Interaction of Species Traits and Environmental Disturbance Predicts Invasion Success of Aquatic Microorganisms. *PLoS ONE,* **7,** e45400.

R Development Core Team (2016) R: A language and environment for statistical computing. Version 3.3.2. R Foundation for Statistical Computing, Vienna, Austria.

Schindler, M.H. & Gessner, M.O. (2009) Functional leaf traits and biodiversity effects on litter decomposition in a stream. *Ecology,* **90,** 1641-1649.

Srivastava, D.S. & Bell, T. (2009) Reducing horizontal and vertical diversity in a foodweb triggers extinctions and impacts functions. *Ecology Letters,* **12,** 1016-1028.

Srivastava, D.S., Cardinale, B.J., Downing, A.L., Duffy, J.E., Jouseau, C., Sankaran, M. & Wright, J.P. (2009) Diversity has stronger top-down than bottom-up effects on decomposition. *Ecology,* **90,** 1073-1083.

Stocker, D., Falkner A.J., Murray, K.M., Lang, A.K., Barnum, T.R., Hepinstall-Cymerman, J., Conroy, M.J., Cooper, R.J. & Pringle C.M. 2017. Decomposition of terrestrial resource subsidies in headwater stream: Does consumer diversity matter? *Ecosphere*, **8**, e01868.

Vos, V.C.A., van Ruijven, J., Berg, M.P., Peeters, E.T.H.M. & Berendse, F. (2013) Leaf litter quality drives litter mixing effects through complementary resource use among detritivores. *Oecologia,* **173,** 269-280.

Wardle, D.A., Bonner, K.I. & Nicholson, K.S. (1997) Biodiversity and Plant Litter: Experimental Evidence Which Does Not Support the View That Enhanced Species Richness Improves Ecosystem Function. *Oikos,* **79,** 247-258.

Webster, J.R. & Benfield, E.F. (1986) Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology, Evolution and Systematics,* **17,** 567-594.

Woodward, G., Gessner, M.O., Giller, P.S., Gulis, V., Hladyz, S., Lecerf, A., Malmqvist, B., McKie, B.G., Tiegs, S.D., Cariss, H., Dobson, M., Elosegi, A., Ferreira, V., Graça, M.A.S., Fleituch, T., Lacoursière, J.O., Nistorescu, M., Pozo, J., Risnoveanu, G., Schindler, M., Vadineanu, A., Vought, L.B.M. & Chauvet, E. (2012) Continental-scale effects of nutrient pollution on stream ecosystem functioning. *Science,* **336,** 1438-1440.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Tables

Table 1: Leaf litter composition (Nitrogen N, Phosphorus P, Carbon to Nitrogen C:N, Carbon to Phosphorus C:P and Nitrogen to Phosphorus N:P ratios) of the leaf litter species used in the experiments.

Leaf type	N content (mg N/g dry weight, mean±sd)	P content (mg P/g dry weight, mean±sd)	C:N atomic ratio (mean±sd)	C:P atomic ratio (mean±sd)	N:P atomic ratio (mean±sd)
Alnus	23.94±4.63	0.799±0.156	20.90±4.68	1386.19±326.53	66.59±7.71
Fagus	7.24±2.37	0.373±0.023	69.22±16.63	2798.77±196.72	43.09±14.63
Populus	10.99±4.34	0.725±0.091	43.98±12.37	1363.56±160.31	34.22±14.97
Quercus	6.58±0.89	0.467±0.113	73.85±11.30	2380.66±608.91	32.08±5.68

Table 2: GLM on the effect of the decomposer community and the type of resource (leaf litter type/combination) on litter decomposition.

Source	Df	Deviance	Resid. Df	Resid. Dev	F-value	P-value
Community	1	4.89	98	75.06	5.98	0.016
Resource Type	10	91.93	99	79.95	11.25	< 0.0001
Interaction	10	4.37	88	70.70	0.53	0.86
NULL			109	171.89		

Table 3: Summary of linear mixed models used to test for effects of the decomposer community, the resource type and their interaction on several response variables. Dissolved oxygen concentration, density of protists, microbial biomass, median cell size and bacterial density were used as response variables. Fixed effects were tested with F-tests, which test for differences in means, whereas random effects were tested with Chi^2-tests, which test for independency.

Response Variable	Source	Df	Den Df	F-/χ^2-value	P-value
Oxygen Concentration	**Community**	1	84	6.29	0.014
	Resource Type	10	84	17.61	< 0.0001
	Interaction	10	84	0.51	0.88
	Day	-	-	2079.3	< 0.0001
Density	**Community**	1	84	0.004	0.95
	Resource Type	10	84	6.05	< 0.0001
	Interaction	10	84	0.27	0.99
	Day	-	-	134.25	< 0.0001
Biomass	**Community**	1	84	0.07	0.79
	Resource Type	10	84	6.95	< 0.0001
	Interaction	10	84	0.67	0.75
	Day	-	-	29.4	< 0.0001
Median size	**Community**	1	84	2.99	0.09
	Resource Type	10	84	1.32	0.23
	Interaction	10	84	0.55	0.85
	Day	-	-	434.22	< 0.0001
Bacterial Density	**Community**	1	84	2.63	0.11
	Resource Type	10	84	3.80	0.0003
	Interaction	10	84	0.57	0.84
	Day	-	-	548.3	< 0.0001

Figure legends

Fig. 1. Experimental setup. We had 11 communities of different leaf litter diversities (*Alnus*, *Fagus, Populus* and *Quercus* leaves as single species, all possible 2-species and the 4-species combinations) that were exposed to complete and size-fractionated decomposer communities, each combination replicated five times.

Fig. 2: Decomposed leaf litter (mean±se percentage of initial total litter dry biomass) of different litter types and their combinations at the end of the experiment (day 72). Colors indicate single species leaf litter treatments (green = *Alnus*, blue = *Fagus,* pink = *Populus*, orange = *Quercus)*, light grey is used for all possible pairwise combinations of the leaf litter species, and dark grey indicates the four-species leaf litter combination; all treatments are also labelled by the species name first-letter abbreviation. The horizontal red lines give expected additive values (mean across the respective single species treatments). Two different decomposer communities were used: (a) a natural, complete decomposer community (filled bars) and (b) a size-fractionated decomposer community (dashed bars).

Fig. 3: Decomposed leaf litter (mean±se percentage of initial litter dry biomass) of different litter types at the end of the experiment (day 72). For each of the four leaf litter species (*Alnus, Fagus, Populus*, and *Quercus*), their biomass loss is given either when they were in single-species microcosms, in two-species combinations or in the four-species combination. The decomposer community was either a complete decomposer community (solid bars; a–d) or a size-fractionated decomposer community (dashed bars; e–h).

Fig. 4: Average concentrations of dissolved oxygen (mean±se) across the whole experiment. Each line represents oxygen concentrations from microcosms with the single leaf litter species treatments as resource types (green = *Alnus*, blue = *Fagus,* pink = *Populus*, orange = *Quercus*). Solid lines indicate complete microbial decomposer communities (a) and dashed lines represent size-fractionated decomposer communities (b).

Fig. 5: Temporal variation of decomposer community metrics (CASY cell counter data of mostly eukaryotic microbial communities; mean±se) across the whole experiment. Panels show densities (cell counts ml^{-1}; a, b), living biomass (µg ml^{-1}; c, d) and median cell size distribution (µm; e, f). Each line represents values from microcosms with the different single leaf litter species treatments (green = *Alnus*, blue = *Fagus,* pink = *Populus*, orange = *Quercus*). Solid lines indicate complete decomposer communities (a, c, e) and dashed lines represent size-fractionated communities (b, d, f).

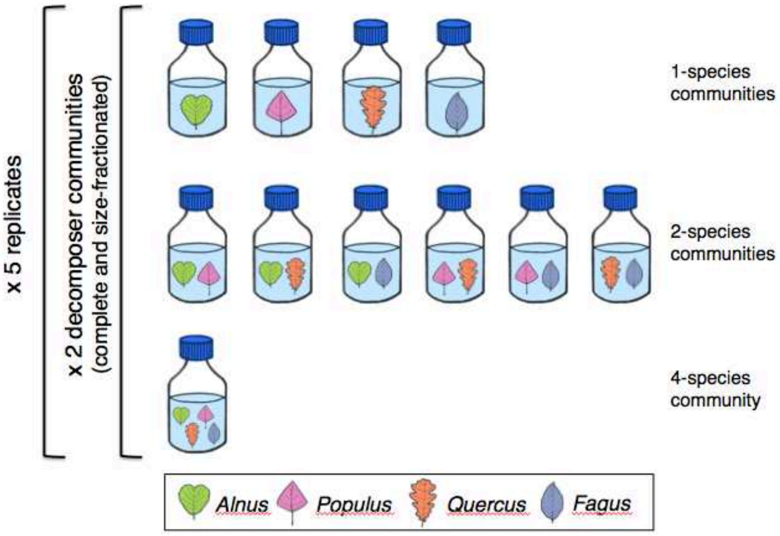

x 5 replicates
x 2 decomposer communities
(complete and size-fractionated)
1-species
communities
2-species
communities
4-species
community
Alnus
Populus
Quercus
Fagus

Figure 1

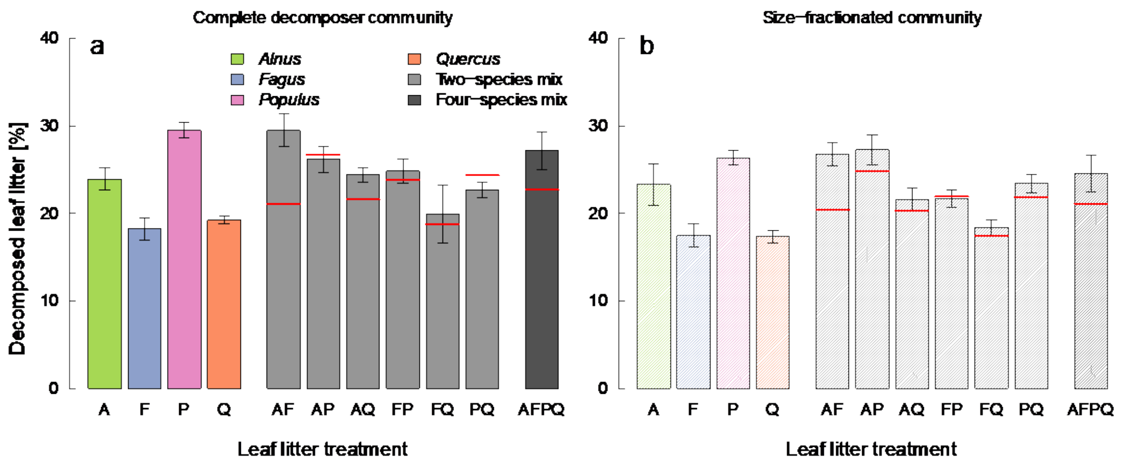
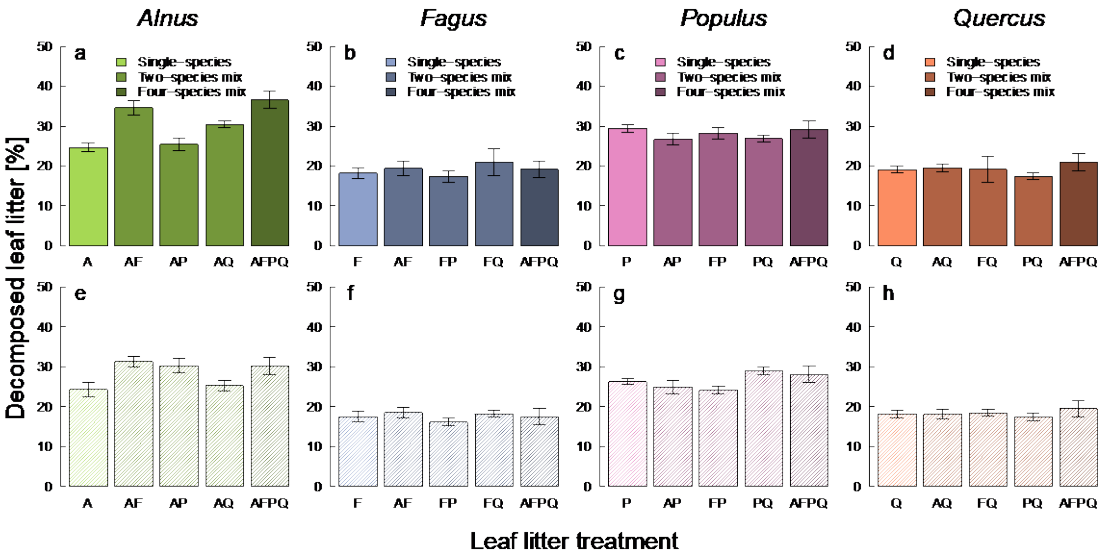

Complete decomposer community
a
Alnus
Fagus
Populus
Quercus
Two−species mix
Four−species mix
Decomposed leaf litter [%]
0
10
20
30
40
A F P Q AF AP AQ FP FQ PQ AFPQ
Leaf litter treatment
Size−fractionated community
b
0
10
20
30
40
A F P Q AF AP AQ FP FQ PQ AFPQ
Leaf litter treatment

Figure 2



705

706 **Figure 3**

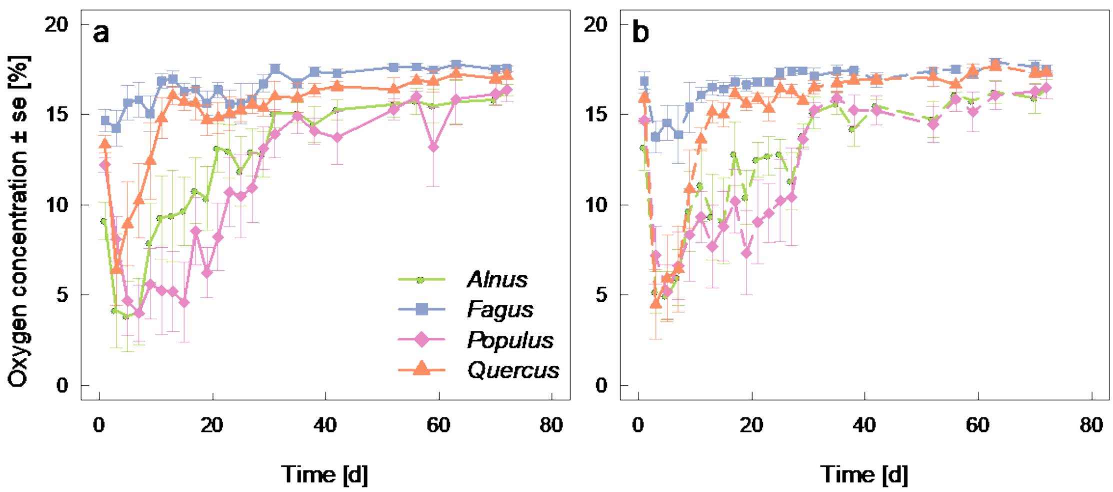

a
b
Oxygen concentration ± se [%]
Time [d]
Time [d]
Alnus
Fagus
Populus
Quercus

Figure 4

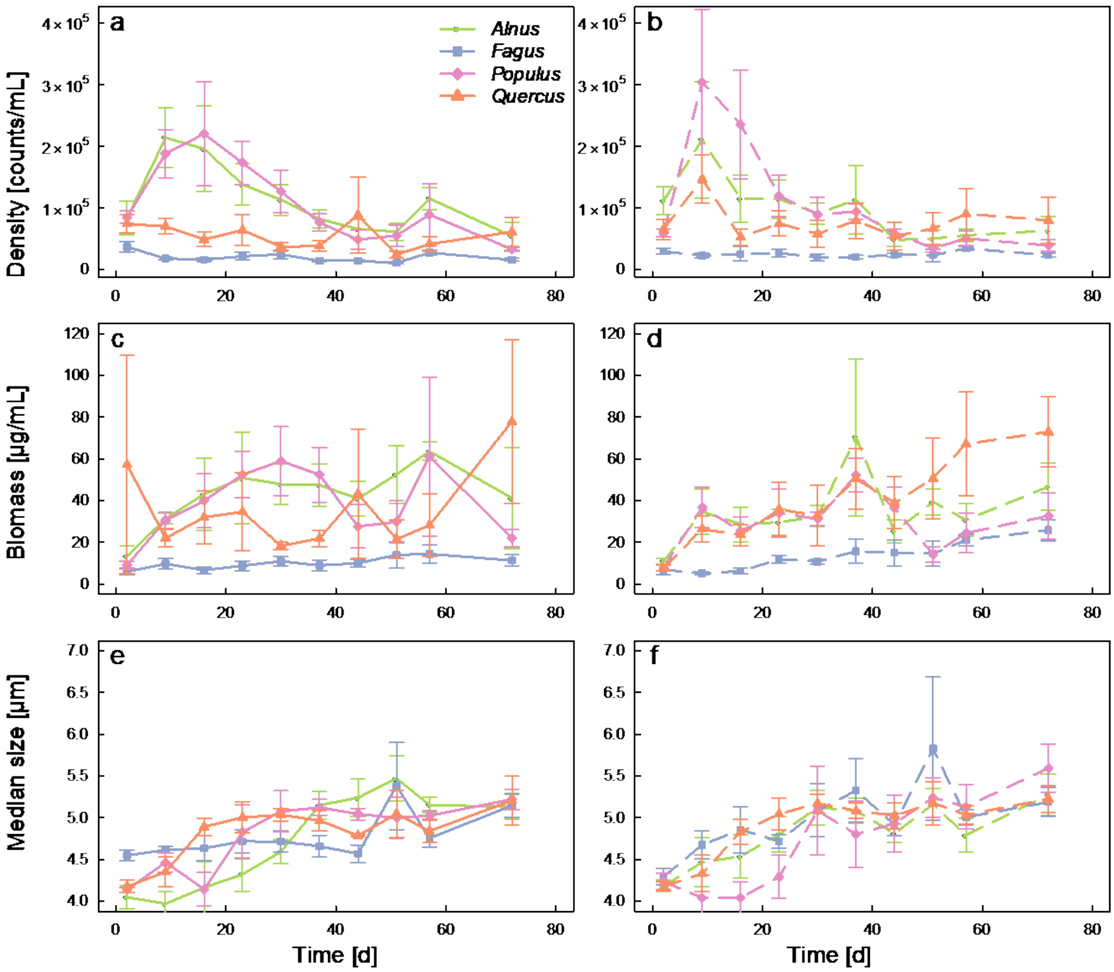

a
b
c
d
e
f
Alnus
Fagus
Populus
Quercus
Density [counts/mL]
Biomass [µg/mL]
Median size [µm]
Time [d]
Time [d]

Figure 5